Plasmodium falciparum Sporozoite ELISA Reagent Kit

Catalog No. MRA-890

This reagent is the tangible property of the U.S. Government.

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

CDC, Atlanta, Georgia, USA

Product Description:

This enzyme linked immunosorbent assay (ELISA) kit is used to detect malaria-infected mosquitoes by utilizing monoclonal antibodies specific for *Plasmodium falciparum* (Pf) circumsporozoite proteins. MRA-890 was authenticated by the contributor. For detailed instructions on the use of this kit, please refer to the ELISA Directions attached to this Product Information Sheet.[1]

Material Provided:

Each kit of MRA-890 contains three vials, each containing purified, lyophilized monoclonal antibody: capture Pf2A10-CDC, peroxidase-labeled Pf2A10-CDC and Pf-PC positive control.

Packaging/Storage:

MRA-890 is provided at ambient temperature. Lyophilized material is stable at ambient temperature or 4°C only for short periods. It should be stored at -20°C or colder immediately upon arrival.

Reconstitution/Storage:

Each vial label will list the amount of glycerol:water to be added. A glycerol:water (1:1) solution is used to reconstitute lyophilized material and allows for storage at -20°C without freeze-thawing. This step only needs to be performed when a new vial of capture or conjugate needs to be reconstituted. Freeze-thaw cycles should be avoided. Do not add sodium azide to solutions as it is a peroxidase inhibitor. Do not add thimerosal. Store at -20°C once reconstituted.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Plasmodium falciparum* Sporozoite ELISA Reagent Kit, MRA-890, contributed by Robert A. Wirtz."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.

Disclaimers:

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References:

1. Methods in *Anopheles* Research, section 8.2.

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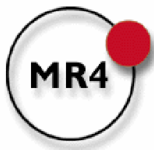

MALARIA RESEARCH & REFERENCE REAGENT RESOURCE CENTER

PLASMODIUM CIRCUMSPOROZOITE ELISA DIRECTIONS

MRA-890 and MRA-1028K

Revised August 2022

Enzyme-linked immunosorbent assays (ELISAs) were developed to detect *Plasmodium falciparum*, *P. vivax*-210 and *P. vivax*-247 circumsporozoite (CS) proteins in malaria infected mosquitoes (Burkot et al, 1984; Collins et al, 1985; Rosenberg et al, 1989; Wirtz et al, 1986, 1987 and 1992). The sensitivity and specificity of the ELISAs are based on the monoclonal antibodies (mAbs) used in the assay. CS protein can be present in the developing oocysts, dissolved in hemolymph and on sporozoites in the hemocoel or salivary glands. A positive CS-ELISA on a mosquito does not establish that species as a vector and results may not be synonymous with salivary gland sporozoite dissections.

The CS-ELISAs can be carried out on fresh, frozen, or dried mosquitoes. If specimens are to be dried, they must be processed quickly and kept dry (stored with desiccant) to prevent microbial growth, which can cause high background values in the CS-ELISAs. Before collection of the mosquitoes is initiated, consideration should be given to the possibility of conducting other tests (e.g., molecular assays, host blood meal ELISAs, etc.) that may require different storage conditions or extraction buffers. Voucher specimens should also be collected and saved.

The "sandwich" ELISA begins with adsorption of the capture mAb to the wells of a microtiter plate (Fig. 1A). After this, the well contents are aspirated and the remaining binding sites are blocked with blocking buffer. Mosquito specimens are ground in blocking buffer containing IGEPAL CA-630 and an aliquot is placed in wells for testing. Positive and negative controls are also added to specific plate wells at this time. If CS antigen is present (depicted as diamond in Fig. 1B) it will form an antigen-antibody complex with the capture mAb. After incubating for 2 hours at room temperature, the mosquito triturate is aspirated and the wells are washed. Peroxidase-labeled conjugate mAb is then added to the wells, completing the formation of the "sandwich" (Fig. 1C). After 1 hour, the well contents are aspirated, the plate is washed again and the clear ABTS substrate solution is added (Fig. 1D). As the peroxidase enzyme reacts with the substrate, a dark green product is formed (Fig. 1D), the intensity of the color is proportional to the amount of CS antigen present in the test sample. Results are read visually or at 405-414nm using an ELISA plate reader 30 minutes after the substrate has been added.

The assay involves 2 steps:

1. Screening phase – ELISA is used to identify positive samples.
2. Boiled retests – samples determined to be positive in the screening phase should be boiled and retested. An aliquot of homogenate is removed, heated at 100°C for 10 minutes and re-tested. Only samples that test positive during the screening AND boiled retest assays are considered positive.

The boiled retest step can be performed using a heat block, thermal cycler or boiling water and will help to eliminate false positive caused by heat-unstable cross-reactive proteins (Durnez et al, 2011).

If it is necessary to test for all three *Plasmodium* species (Pf, Pv210 and Pv247), assays can be run concurrently on 3 plates. If there is not a large enough sample volume, the 3 assays may be run consecutively (Fig. 2), "recycling" the sample by transferring it to another plate coated with a different antibody.

Note that distribution of new *P. vivax*-210 and 247 recombinant positive control antigens began in September 2009. The amounts contained in mAb and positive control vials are subject to change. Any reagents received should be used with the protocol version included in the kit. The optimization that was performed to establish this protocol was conducted under specific conditions with specific supplies (see page 11). Further optimization may be necessary, considering your lab conditions or with alternative supplies.

Sets of CS-ELISA reagents can be requested through BEI Resources. All other supplies and reagents used in the CS-ELISAs are available from commercial sources. For technical advice, a more detailed protocol or recommendations regarding the use of this kit, contact:

Alice Sutcliffe, MS
CDC/CGH/DPDM/EB
Email: ASutcliffe@cdc.gov
Phone: 404-718-4326

Adeline Chan, Ph.D.
CDC/CGH/DPDM/EB
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Phone: 404-718-4305

For a step-by-step video protocol on preparing reagents and performing the assay, visit: https://cfwe.auburn.edu/cselisa-training/

Figure 1. The “sandwich” ELISA for detection of *Plasmodium falciparum* and *P. vivax* circumsporozoite proteins.

A. Anti-sporozoite monoclonal “capture” antibody adsorbed to well.

B. Mosquito homogenate added to well.

C. Anti-sporozoite monoclonal peroxidase-labeled conjugate antibody added to well.

D. ABTS substrate added to well.

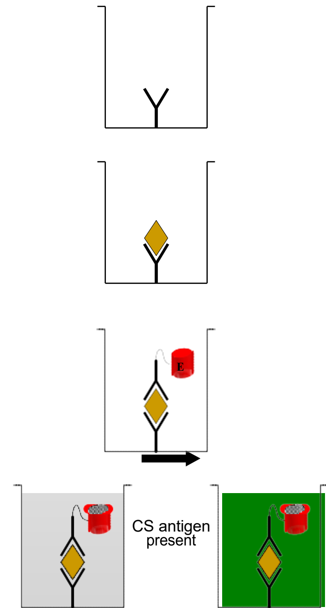
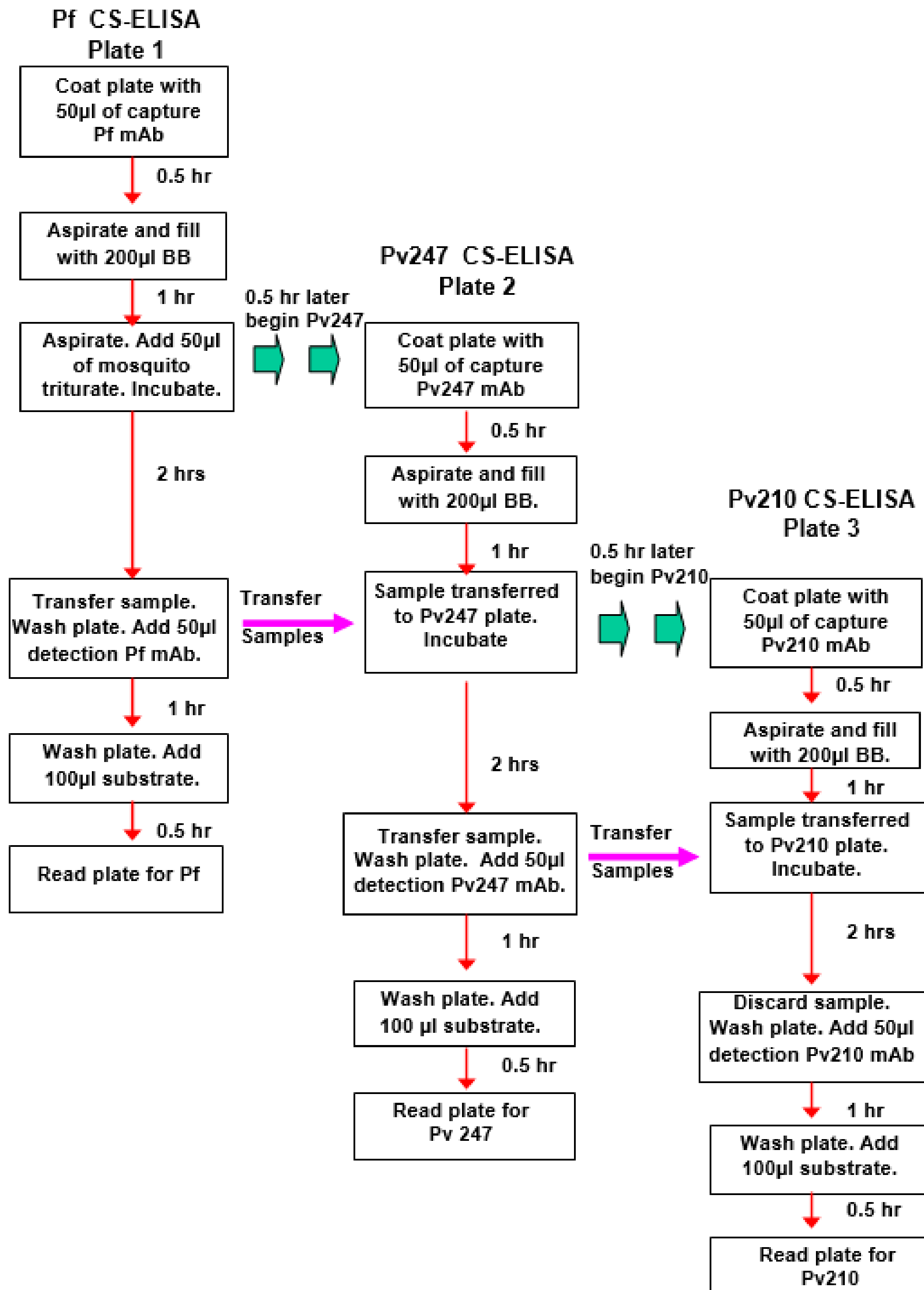
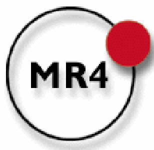


Figure 2. Scheme for running all three *Plasmodium* species (Pf, Pv210 and Pv247) consecutively. This allows recycling of sample by transferring it to another plate containing different antibody.





PREPARATION OF SPOROZOITE ELISA SOLUTIONS

For a step-by-step video protocol on preparing reagents and performing the assay, visit:
https://cfwe.auburn.edu/cselisa-training/

The following solutions should be prepared prior to performing the procedure.

1. Phenol Red – A stock solution of phenol red can be made. This eliminates the need to weigh out small amounts of this material. Phenol red added to solutions is only used as a dye to aid in visualization and is optional. Use caution when handling phenol red as it will stain skin and clothes.

(1) Weigh 1g of phenol red into a tube that can hold at least 10ml.
(2) Add 10ml of water to the measured phenol red. The concentration will be 100mg/ml.
(3) Cap the tube and gently vortex until the phenol red is dissolved.
(4) Briefly centrifuge the tube to collect the solution at the bottom of the tube.
(5) Prepare 1ml aliquots of the solution and store at 4ºC.

2. 1X Phosphate Buffered Saline (PBS), 10mM, pH 7.4 – Use stock laboratory PBS.

(1) OPTIONAL: Add 100µL of 100mg/ml phenol red stock solution. The concentration of phenol red in PBS will be 10mg/L.
(2) Shake to mix well.
(3) Store at 4ºC. Shelf life has not been determined.

3. Blocking Buffer (BB) – Use only ELISA grade Sigma casein (Sigma C7078).

	Batch Volume	
	500 ml	**1000 ml**
Casein	2.5 g	5.0 g
NaOH, 0.1N	50.0 ml	100.0 ml
1X PBS, 10mM pH 7.4 (no phenol red)	450.0 ml	900.0 ml
OPTIONAL: Phenol Red Solution, 100mg/ml	100 µl	200 µl

(1) Bring 0.1 N NaOH to a boil in a flask with a stir bar mixing on low.
(2) Slowly add the casein and mix until dissolved in 0.1N NaOH.
(3) Remove flask from heat and allow solution to cool to room temperature with stir bar mixing on low.
(4) Slowly add the PBS with stir bar mixing on low.
(5) Adjust the pH to 7.4 with 1N HCl with stir bar mixing on low.
(6) OPTIONAL: Add the phenol red with stir bar mixing on low. The concentration of phenol red in BB will be 20mg/L.
(7) Store at 4°C for up to 1 week or aliquot into 50ml tubes for long term storage at -20°C.

4. Grinding Buffer – Blocking buffer with IGEPAL CA-630: Shelf life at 4°C is 1 week.

(1) Combine 25ml of BB and 125µl of Igepal CA-630. This will be sufficient for approximately one plate.
(2) Mix well, using a vortex, to dissolve the Igepal CA-630 in the BB.
(3) Store at 4°C for up to 1 week.

5. 1X Phosphate Buffered Saline-Tween (PBS-T) Wash Solution pH 7.4 – 10mM PBS plus 0.05% Tween 20.

(1) Add 500µl Tween 20 to 1 liter of PBS (no phenol red).
(2) OPTIONAL: Add 100µL of 100mg/ml phenol red stock solution. The concentration of phenol red in PBS-tween will be 10mg/L.
(3) Shake to mix well.
(4) Store at 4ºC . Shelf life has not been determined.

6. Glycerol:Water Solution – A 1:1 glycerol:water solution is used to rehydrate lyophilized antibodies. This solution allows for storage at -20°C without freeze-thawing and minimizes deterioration of antibodies due to freeze-thawing.

(1) Add 5ml of reagent grade water to a tube that that can hold at least 10ml
(2) Add 5ml of reagent grade glycerol to the water. Glycerol is very viscous and should be pipetted slowly and with a wide-bore pipette tip, if possible.
(3) Store at room temperature. Shelf life has not been determined.

7. Monoclonal Antibody (mAb) Stock Solution: Monoclonal antibody capture and conjugate will be shipped lyophilized and should always be stored at -20°C. The label will list the amount of glycerol:water to be added and the res µlting stock concentration should be 0.5 mg/ml. This step only needs to be performed when a new vial of peroxidase-labeled conjugate antibody is being started. Horseradish peroxidase is photo-sensitive so these antibodies should be stored in dark or foil-wrapped containers.

(1) Determine the volume of 1:1 glycerol:water that needs to be added to the lyophilized mAb to achieve a final concentration of 0.5mg/ml.
(2) Gently pipette the calculated volume into the vial containing lyophilized mAb
(3) Allow the contents of the vial to dissolve at 4ºC for 10 minutes or longer.
(4) Gently mix the vial using a vortex at low setting or by inverting the vial several times.
(5) Briefly centrifuge the vial to collect the solution at the bottom of the vial.
(6) OPTIONAL: Prepare 500µl aliquots.
(7) Store at -20°C. Shelf life has not been determined.

8. Positive controls (PC): Positive controls received will be lyophilized and should always be stored at -20°C. All controls should be rehydrated with 1000 µl of blocking buffer and the resulting stock concentration will vary with each positive control. This step only needs to be performed when a new vial of positive control is reconstituted. Pv210 and Pv247 positive controls are a combined, recombinant protein provided by Protein Potential.

(1) Gently pipette 1000µl of BB into the vial containing lyophilized PC
(2) Allow the contents of the vial to dissolve into solution at 4ºC for 10 minutes or longer.
(3) Gently mix the vial using a vortex at low setting or by inverting the vial several times.
(4) Briefly centrifuge the vial to collect the solution at the bottom of the vial.
(5) OPTIONAL: Prepare 200µl aliquots.
(6) Store at -20°C.
(7) Use the tables below to determine the volume of stock PC to add to BB to create the "Working" tube for use directly on each ELISA plate

Tube	Vol BB		Pf		Pf Conc.
Stock	1000 µl	+	0.1 µg lyophilized control	=	100 pg / µl
Working	490 µl	+	10 µl rehydrated stock control	=	2 pg / µl

Tube	Vol BB		Pv210		Pv-210 Conc.
Stock	1000 µl	+	9.1 µg lyophilized control	=	9.1 ng / µl
Working	490 µl	+	10 µl rehydrated stock control	=	182 pg / µl

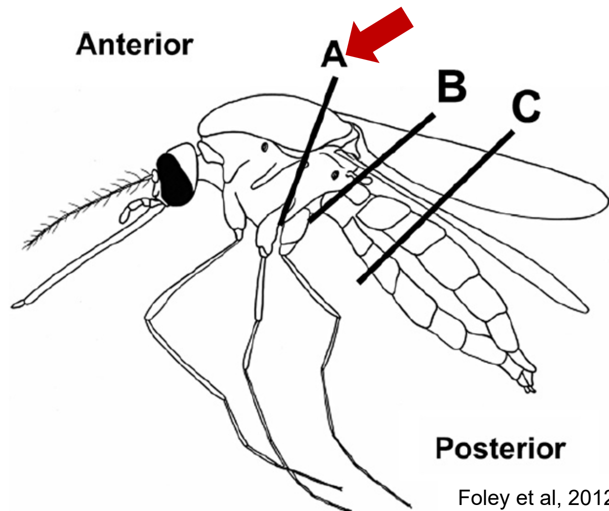
Tube	Vol BB		Pv247		Pv-247 Conc.
Stock	1000 µl	+	4.55 µg lyophilized control	=	4.55 ng / µl
Working	490 µl	+	10 µl rehydrated stock control	=	91 pg / µl

9. Negative Controls
The ideal negative controls are head and thoraces from female mosquitoes that have never taken a *Plasmodium* spp. infected bloodmeal and that have been stored and prepared in the same manner as test samples (ie. fresh, frozen, desiccated, etc.). If these are not available, head and thoraces from male mosquitoes can be used.

10. Mosquito Sample Preparation.
It is recommended samples be prepared the day before the CS-ELISA is to be performed.

(1) Using a scalpel, carefully dissect the mosquito head and thorax from the abdomen across position A (Figure 3). Dissecting below this point may lead to false positives (Foley et al, 2012). Wings and legs should also be removed and excluded from the CS-ELISA homogenate.
(2) Place a single head and thorax or pooled head and thoraces of no more than 10 mosquitoes in a labeled 1.5ml centrifuge grinding tube.
(3) Add 50µl of Grinding Buffer.
(4) Grind well using a pestle.
(5) Rinse the pestle twice, each time with 100µl of Grinding Buffer, catching the rinses in the tube containing the mosquito triturate. The final volume will be approximately 250µl.
(6) Store at 4ºC for 24 hours or -20ºC for periods longer than 24 hours.
(7) Rinse the pestle in PBS-Tween twice and dry with tissue to prevent contamination between mosquitoes.

Figure 3. Bisection position for proper mosquito preparation for CS-ELISA

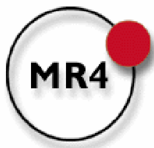


11. ABTS Substrate Solution
The substrate solution is made as a 1:1 dilution of ABTS solution A to solution B (SeraCare 5120-0032). At the final step of the ELISA, 100 µl of substrate solution is added to each well. ABTS is photo-sensitive so should be stored in dark or foil-wrapped containers until use. To fill each of the 96 wells on a plate with 100µl requires 9.6ml. It is convenient to make up 10.0ml of ABTS solution at the appropriate step in the protocol.

(1) Combine 5mL of solution A and 5mL of solution B in tube
(2) Vortex to mix well.
(3) Store the mixture wrapped in foil at 4°C until use.

NOTES AND TROUBLESHOOTING FOR CS-ELISA

1. Do not add sodium azide to solutions as it is a peroxidase inhibitor.
2. We no longer add thimerosal to solutions. Thimerosal is mercury-based and presents problems with proper disposal.
3. Store all solution as described when not in use and adhere to shelf lives to prevent problems related to microbial growth in working solutions. If information regarding shelf life is not described, the shelf life has not been determined.
4. Order supplies from the suggested vendors or ensure that they are identical to those recommended (see Sporozoite ELISA Supplies on page 11). Be aware too that different 1.5ml tubes have internal dimensions in which the pestles may not work, microtiter plates have different binding qualities, and different caseins are less efficient in blocking and may result in decreased sensitivity or higher background OD values.
5. Cover plate during incubations to prevent evaporation. Incubate plate in the dark (especially for incubation of peroxidase-labeled conjugate and substrate solution). An efficient way of doing this is to use a small cardboard box lid or to line the lid of a pipette tip box with aluminum foil.
6. For Pv210 and Pv247 species, it may be necessary to read plates at 30 and 60 minutes. Thirty minutes may not be sufficient for a reliable color change. For Pf species, a 30-minute incubation should be sufficient.
7. Only high, lab grade paper towels should be used while performing CS-ELISA testing, particularly to “bang” the ELISA plate on, for removing excess solutions. Use of brown paper towels and kitchen paper towels should be avoided as the result is high background values. It is thought that fibers from these towels adhere to the plates and interfere with detection. If high, lab grade paper towels are not available, perform this step over the sink or ensure careful removal of all solutions with a vacuum system for each required step.
8. Do not vortex samples as this can lead to high background and inaccurate absorbance values. Short centrifugation can be used to settle body parts and allow pipetting of a clean sample. This centrifugation will not pellet sporozoites and they will remain suspended in the supernatant.
9. Wipe the bottom of the ELISA plate with ethanol to remove debris and oils from CS-ELISA plate before reading. Fingerprints, oils and debris on the plate can lead to inaccurate absorbance values.
10. The Corning® 2797 96-well clear round bottom PVC (soft plate) cannot be reliably used with certain, especially newer, models of plate readers. This is due to distortion of the plate upon entering the reader carriage or a poor fit of plate in the reader carriage. This can be solved by using Corning® 3366 96-Well polystyrene plates (hard plate; round well, high binding).
11. If a vacuum or aspiration system is not available to empty contents of the ELISA plate, bang plate on sink edge into the sink and then again on paper towels. Do not use brown paper towels.
12. For a step-by step video protocol on preparing reagents and performing the assay, visit: **https://cfwe.auburn.edu/cselisa-training/**



SPOROZOITE ELISA DIRECTIONS

- For a step-by step video protocol on preparing reagents and performing the assay, visit: **https://cfwe.auburn.edu/cselisa-training/**
- Be sure to read the information and notes preceding these directions before beginning.
- Be sure to cover or seal plate during all incubations to prevent evaporation.
- Use a separate plate for each sporozoite species.

(1) Fill out the top portion of the Sporozoite ELISA worksheet (Worksheet 1). Mark the ELISA plate in order to maintain correct plate orientation.

(2) Prepare a working solution of mAb capture by adding **PBS** to the reconstituted capture mAb based on the volumes by species listed below. Vortex gently.

Species	µg / 5 ml	µl capture mAb stock/5 ml
Pf	20 µg	40 µl stock + 5 ml PBS
Pv-210	10 µg	20 µl stock + 5 ml PBS
Pv-247	10 µg	20 µl stock + 5 ml PBS

(3) Place 50µl of mAb solution made in step 2 in each well of the corresponding ELISA plate.

(4) Cover plate and incubate for **30 MINUTES** at room temperature.

(5) Aspirate well contents and bang plate upside down on paper towel 5 times, holding sides only.

(6) Fill wells with 200µl BB

(7) Cover plate and incubate for **1 HOUR** at room temperature.

   a. During this incubation, positive controls should be prepared, see page 5 for instructions.

(8) Aspirate well contents and bang plate upside down on paper towel 5 times holding sides only.

(9) Load samples and controls into the plate

   a. Add 50µl of positive control from "Working Tube" (see page 5) to well A1.
   b. Add 50µl of negative control to wells B1-H1.
   c. Add 50µl of mosquito triturate per well to remaining wells.

(10) Cover plate and incubate for **2 HOURS** at room temperature.

(11) Prepare substrate by mixing Substrate A and Substrate B at a 1:1 ratio. A full 96-well plate will require 5ml of Substrate A + 5ml of Substrate B

(12) Prepare a working solution of peroxidase-labeled conjugate mAb by adding BB to the reconstituted conjugate mAb based on the volumes by species listed below. Vortex gently.

Species	µg / 5 ml	µl conjugate mAb stock/5 ml
Pf	5.0 µg	10 µl stock + 5 ml BB
Pv-210	5.0 µg	10 µl stock + 5 ml BB
Pv-247	5.0 µg	10 µl stock + 5 ml BB

(13) Check enzyme activity by mixing 5µl of the mAb conjugate made in step 12 with 100µl of the ABTS substrate made in step 11 in a separate tube. Vortex gently. There should be rapid color change indicating that the peroxidase enzyme and the substrate are functional.

(14) Aspirate well contents and bang plate upside down on paper towel 5 times holding sides only.

(15) Wash wells 2 times with 200µl of PBS-Tween, aspirating and banging plate 5 times with each wash

(16) Add 50µl of peroxidase-labeled conjugate solution made in step 12 to each well.

(17) Cover plate and incubate for **1 HOUR** at room temperature

(18) Aspirate well contents and bang plate upside down on paper towel 5 times holding sides only.

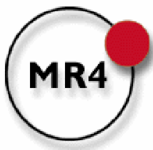
(19) Wash wells 3 times with 200µl of PBS-Tween, aspirating and banging plate 5 times with each wash.

(20) Add 100µl ABTS substrate solution per well prepared in step 11.

(21) Cover plate and incubate for **30 MINUTES** at room temperature.

(22) Handle plate carefully to avoid splashing. Read visually, or at 405-414nm

(23) Following the initial screening of all mosquitoes: determine positive samples, transfer a 50µl aliquot of un-tested sample homogenate to a new tube, heat for 10 minutes at 100°C using a heat block, thermal cycler, boiling water or equivalent and retest. Ensure tube caps are well sealed so they do not pop open during heating steps.



WORKSHEET: ELISA TEMPLATE FOR SCREENING

ELISA Plate No:______________ **DATE:** _______________

Capture mAb: Lot #____________ Peroxidase-labeled conjugate mAb: Lot #:____________

Positive control: Lot#____________

Controls ◄——— Samples ———►

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	**Pos**											
B	**Neg**											
C	**Neg**											
D	**Neg**											
E	**Neg**											
F	**Neg**											
G	**Neg**											
H	**Neg**											

____ 1) Coat plate with **50µl** capture mAb.

→ **0.5 hr incubation**

____ 2) Aspirate wells; fill wells with **200µl** blocking buffer (BB).

→ **1 hr incubation**

____ 3) Aspirate wells; Add **50µl** mosquito triturate and controls

→ **2 hr incubation**

____ 4) Aspirate and wash two times with 200µl PBS-0.05% Tween 20.

____ 5) Add **50µl** peroxidase-mAb. Mix ABTS substrate (1:1).

→ **1 hr incubation (in the dark)**

____ 6) Aspirate and wash 3 times with 200µl PBS-0.05% Tween 20.

____ 7) Add **100µl** substrate: ____ a) Enzyme check; ____ b) 100µl/well.

→ **0.5 hr incubation (in the dark)**

____ 8) Read absorbency 405nm.

Analysis:
Calculate the cut-off = 2 x mean absorbance values of negative samples.
Positive (RETEST) = Samples which have absorbance values above the cut-off.
Negative = Samples which have absorbance values below the calculated cut-off.

WORKSHEET: ELISA TEMPLATE FOR BOILED RE-TESTING

ELISA Plate No:______________ **DATE:** _______________

Capture mAb: Lot #____________ Peroxidase-labeled conjugate mAb: Lot #:____________

Positive control: Lot#____________

Controls ← **Samples** →

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		**Pos**										
C		**Neg**										
D		**Neg**										
E		**Neg**										
F		**Neg**										
G		**Neg**										
H												

Leave wells around the edge of the plate empty to avoid potential "edge effect" (artifically high absorbance values that can occur around the edge of some 96-well assay plates)

____ 1) Coat plate with **50µl** capture mAb.

→ **0.5 hr incubation**

____ 2) Aspirate wells; fill wells with **200µl** blocking buffer (BB).

→ **1 hr incubation**

____ 3) Aspirate wells; Add **50µl** mosquito triturate and controls

→ **2 hr incubation**

____ 4) Aspirate and wash two times with 200µl PBS-0.05% Tween 20.

____ 5) Add **50µl** peroxidase-mAb. Mix ABTS substrate (1:1).

→ **1 hr incubation (in the dark)**

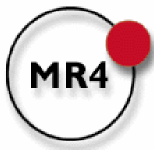
____ 6) Aspirate and wash 3 times with 200µl PBS-0.05% Tween 20.

____ 7) Add **100µl** substrate: ____ a) Enzyme check; ____ b) 100µl/well.

→ **0.5 hr incubation (in the dark)**

____ 8) Read absorbency 405nm.

Analysis:
Calculate the cut-off = 2 x mean absorbance values of negative samples.
Positive = Samples which have absorbance values above the cut-off in both the initial test and boiled-retest
Negative = Samples which have absorbance values below the calculated cut-off.



SPOROZOITE ELISA SUPPLIES

The following is a list of the consumable supplies and reagents recommended for use in the CS-ELISA. Additional equipment needed to conduct ELISAs includes: pH meter, stirring hot plate, pipettes/tips, forceps, scalpels, flasks, test tubes, graduated cylinders, a plate washing system and plate reader.

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SUPPLY	SUPPLIER	CATALOG #
Phenol Red	Fisher Scientific	AAB2171009
Igepal CA-630	Fisher Scientific	ICN19859650
PBS	Fisher Scientific	BP2944100
Tween20	Fisher Scientific	BP337-100
NaOH	Fisher Scientific	AC124260010
HCl	Fisher Scientific	SA48-500
Glycerol	Fisher Scientific	BP2291
Soft ELISA plates (Costar 2797)	Fisher Scientific	07-200-99
Hard ELISA plates (Costar 3366)	Fisher Scientific	07-200-640
Casein	SigmaAldrich	C7078
ABTS 2-component substrate	SeraCare/LGC Group	5120-0032

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